Redox characteristics of cigarette smoke

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Recent studies from this laboratory have concerned the tumorigenicity of cigarette smoke condensate in animals.[1–4] In closely related investigations, the characteristics of alkylation[5,6] and of enzyme inhibition[7,8] of smoke have also been described. In a continuation of this work the redox characteristics of smoke have now been studied.

Few significant reports have been published on this subject. The reducing capacity of cigarette smoke was reported to be equivalent to about 1·2–4·5mg glucose/g tobacco burned, for various tobacco types, using an alkaline ferricyanide-ferrocyanide redox system.[9] Somewhat lower values were obtained in another study employing the Somogyi method for reducing substances.[10] More recently, reduction of the dye, Blue Tetrazolium, by smoke was reported[11] and employed quantitatively to estimate the degree of retention of particulate matter in the human respiratory tract during smoking.[12] However, none of these studies has included even a superficial description of the overall redox characteristics of smoke.

Buffered, aqueous solutions of whole cigarette smoke, and phases thereof were prepared as described previously.[7] The reactions of these solutions with selected dyes of different E'_o values are shown in the Table. Ascorbate and hydrogen peroxide were employed to obtain total reduction or oxidation of the dyes, respectively. All of these results were obtained by reaction of the solutions with the dyes as rapidly as possible after smoking was completed. Whole smoke (WS) and particulate matter (PM) partially reduced 2,6-dichlorophenol indophenol (E'_o,+219mV) (DCP) and oxidised Toluylene Blue (E'_o,+115mV). Vapour phase (VP) had little visible effect on these dyes.

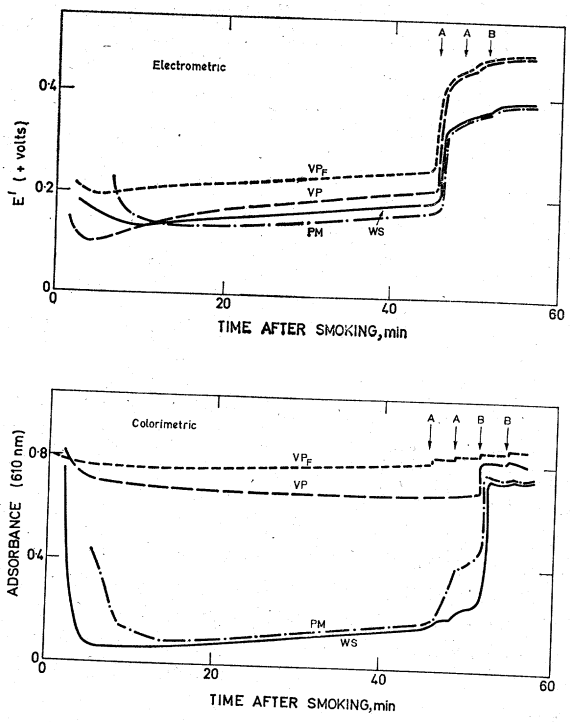
Table
Reaction of solutions of fresh cigarette smoke and phases thereof with selected redox dyes

Addition	*Colour*[a] *DCP* (+219)	*Toluylene blue* (+115)	*Blue tetrazolium* (−80)
None	Blue	Light blue	Tan
Whole smoke	Light blue	Dark blue	Pink
Vapour phase	Blue	Light blue	Light pink
Particulate matter	Light blue	Dark blue	Pink
Ascorbic acid	Colourless	Pink	Bluish-purple
Hydrogen peroxide	Blue	Dark blue	Tan

[a] Solutions of smoke and phases thereof were tested immediately after collection. Protocol: 1ml. smoke solution+1ml. 0·1M phosphate buffer (pH 7·0)+1ml. dye solution. Levels of dyes (mg per tube): DCP and Toluylene Blue, 0·05; Blue Tetrazolium, 1·5mg (in 0·33M sodium hydroxide). DCP=2,6-dichlorophenol indophenol. Figures in parentheses are E'_o values.

Tetrazolium dyes have been employed frequently as histological indicators for dehydrogenases, yielding a dark blue formazan product that is insoluble *in situ*. With this dye, VP undergoes reaction to a slight extent with the dye under the test conditions, and WS and PM produced a pink colour having maximal absorption at 530nm, which has been used by others to determine *in vivo* retention of smoke, as indicated above.[12] However, total reduction of the dye with ascorbate yielded a bluish-purple product with maximal absorption at 610nm. Based on the findings with DCP and Toluylene Blue listed in the Table, reduction of Blue Tetrazolium by smoke would be unexpected owing to the difference in E values. The anomalous colour patterns obtained with smoke may be related to the purity of the commercial dye employed. Recent work by others has shown that commercial *bis*tetrazolium dyes, including Blue Tetrazolium, usually contained small amounts of monotetrazolium salts as contaminants.[13] The latter yield monoformazans on reduction, having absorption maxima 55–80nm lower than the *bis*formazans derived from the corresponding *bis*-salts. In the case of Blue Tetrazolium, the *bis*formazan is blue (λ_{max}.600nm) and the monoformazan is reddish-violet (λ_{max}.545nm).[13] Probably, the monotetrazolium contaminants also have different E'_o values than the *bis*-salts. In the reactions with the smoke solutions, it is possible that smoke components reduced the contaminating mono-salt but did not react with the predominately oxidised form of *bis*-salt in the commercial sample of Blue Tetrazolium. Ascorbate, a strong reducing agent, may have reduced both major constituent and contaminant in the dye, giving a product with the approximate absorption maximum of the diformazan from the *bis*-salt and some alteration in visible colour owing to the contaminating monoformazan. Apparently, the shade of colour developed with ascorbate is also a function of the solubilities of two formazans: the blue component is more soluble in water than in ethanol, and the reverse is true with the reddish-violet compound. However, none of these findings may obviate the proposed use[11] of Blue Tetrazolium to estimate PM or WS, providing the composition of successive commercial batches of dye remains constant.

Phenazine methosulphate reacts with organic compounds



Redox characteristics of cigarette smoke by colorimetric and electrometric determinations. Smoke solutions (1·0ml. equivalent to smoke from 0·1 cigarettes) were added to 2,6-dichlorophenol indophenol solutions (2·0ml. of $2{\cdot}7\times10^{-4}$M) and the absorbance read at the indicated intervals after smoking was completed. Concurrently, electrometric determinations were made under nitrogen as described in the text. A=0·1ml. of 3 per cent hydrogen peroxide added, B=0·05ml. peroxidase solution containing 50μg (12·5 units) added. Absorbances after addition of A and B were corrected for dilution. WS=whole smoke, PM=particulate matter, VP=vapour phase, VP_F=vapour phase filtered through charcoal-acetate type filter

by one electron charge transfer yielding coloured products that are paramagnetic[14,15] A hexane-soluble fraction of smoke condensate which contained no free radicals has been shown to react with PMS to yield a coloured product and a strong e.s.r. signal.[16] In the present study, no visual development of colour was observed with mixtures of PMS and WS or phases thereof. However, a small hypsochromic shift (about 14nm) was observed in the strong maximum of PMS at 386 mμ when VP and PM were present. Ascorbate produced a light green reaction product with PMS and hydrogen peroxide produced no colour change. In general, these results with the smoke solutions appear to be at variance with those discussed above on the hexane-soluble fraction of smoke. Possible reasons for this variance are qualitative and quantitative differences in the smoke components obtained in the employed methods of smoke collection and differences in the studied reaction times.

As shown in the Figure, the reducing capacity of the smoke and phases thereof for DCP was time dependent. A rapid reduction occurred with WS and PM; some slight reduction was also noted with VP after the initial reading. PM appeared to be the major contributor to the reducing capability of WS. In all cases, slow reoxidation occurred after a minimum reducing value was obtained. VP from the smoke of cigarettes containing a multiple activated carbon-cellulose acetate filter[7] showed less reducing capacity than VP from unfiltered smoke. The addition of hydrogen peroxide had a slight effect on VP from both filter and non-filter cigarettes but accelerated the reoxidation step significantly for PM and WS. The addition of peroxidase, which catalyses the oxidation of smoke constituents slowly oxidised by hydrogen peroxide, accelerated markedly the peroxide-smoke reactions from VP (unfiltered smoke), WS and PM.

Electrometric determinations done concurrently on the various solutions used in the colorimetric studies gave the pattern shown in the Figure. Measurements were made using a platinum electrode and silver-silver chloride half cell. The major difference is seen in VP of filtered and unfiltered smoke both of which showed stronger relative reducing capacities in this measurement compared to the colorimetric studies. Also, the terminal oxidation on the addition of hydrogen peroxide produced a more rapid and extensive reaction when electrometric determination was employed. Some of these differences between the colorimetric and electrometric methods may be due to a methodological artifact inherent in colorimetric redox systems: redox dyes act in a manner similar to colorimetric pH indicators in preventing shifts in potential analogous to buffering systems.

All of these results provide further evidence of the dynamic state of fresh cigarette smoke and phases thereof. A variety of reactions between smoke constituents can be visualised, many of which can involve redox reactions. The presence of redox pairs in smoke is evident from a perusal of the more than 1100 known smoke constituents,[17] *e.g.* quinones[18] and dihydric phenols. In addition to redox reactions, reducing groups may be generated in other ways, *e.g.* reaction between nucleophiles and disulphides in smoke, as indicated in recent studies on the scission of 5,5-dithiobis-(2-nitrobenzoic acid) by HCN, H_2S, and so on.[19] Reducing groups may be lost through oxidation or addition reactions with such smoke constituents as the saturated and unsaturated aldehydes[8,19] or by alkylation.[5]

The dynamic state of redox in the cigarette smoke has several implications. First, it demonstrates the rapid reactions which are occurring both within and between the two smoke phases resulting in changes in their composition with time. Secondly, it shows the possibility of coupled reactions between smoke constituents and components in physiological milieu, *e.g.* sulphydryl-disulphide systems and various redox pairs involved in oxidative phorphorylation. Although difficult to measure, the E_o' values for many sulphydryl-disulphide systems are in the range of 0 to −320mV. Oxidation of sulphydryl compounds by constituents of fresh smoke would be expected, and the above redox patterns superficially resemble the course of enzymatic inhibition of smoke which involves deactivation of sulphydryl groups, at least in part.[7,8] Also, the E values for cigarette smoke are in the range of the E_o' of cytochrome C (+260mV) and preliminary results have indicated that WS and VP reduce the pigment *in vitro*.

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